Assessment of the effects of diet and physical rehabilitation on radiographic findings and markers of synovial inflammation in dogs following tibial plateau leveling osteotomy

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OBJECTIVE
To determine the effects of an omega-3 fatty acid and protein–enriched diet, physical rehabilitation, or both on radiographic findings and markers of synovial inflammation in dogs following tibial plateau leveling osteotomy and arthroscopic surgery for treatment of cranial cruciate ligament disease.

DESIGN
Randomized, prospective clinical trial.

ANIMALS
48 dogs with unilateral cranial cruciate ligament disease.

PROCEDURES
Dogs were randomly assigned to receive a dry omega-3 fatty acid and protein–enriched dog food formulated to support joint health (test food [TF]), a dry food formulated for adult canine maintenance (control food [CF]), TF plus rehabilitation, or CF plus rehabilitation after surgery. Synovial fluid prostaglandin (PG) E_2 and interleukin-1β concentrations, radiographic osteoarthritis scores, osteotomy site healing, and patellar ligament thickness were assessed at predetermined time points up to 6 months after surgery.

RESULTS
Dogs that received CF had significantly higher PGE_2 concentrations over time following surgery than did dogs that received TF, regardless of rehabilitation status. Synovial fluid interleukin-1β concentrations did not change over time in any groups. Diet and rehabilitation were both associated with osteoarthritis scores, with significantly lower scores over time for dogs that received TF versus CF and for dogs that underwent rehabilitation versus those that did not. Proportions of dogs with complete osteotomy healing 8 and 24 weeks after surgery were significantly lower for dogs that received TF than for dogs that received CF, regardless of rehabilitation status.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that feeding the TF can result in lower synovial fluid PGE_2 concentrations and that both the TF and rehabilitation can reduce progression of osteoarthritis in the 6 months following tibial plateau leveling osteotomy; clinical relevance of slower osteotomy healing in dogs fed the TF was unclear. (*J Am Vet Med Assoc* 2018;252:701–709)

Dogs with CCL rupture commonly develop osteoarthritis in the affected joint, and attempts to slow osteoarthritis progression with rehabilitation following TPLO have had mixed results.[1–6] However, some previous studies[3,4,7–10] have suggested that rehabilitation protocols can improve various clinical outcome measures following surgical treatment of CCL disease.

ABBREVIATIONS

CCL	Cranial cruciate ligament
CF	Control food
CF-R	Control food plus rehabilitation
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
IL	Interleukin
PG	Prostaglandin
TF	Test food
TF-R	Test food plus rehabilitation
TPLO	Tibial plateau leveling osteotomy

Diets with high omega-3 fatty acid content have also been recommended for management of osteoarthritis in dogs.[11–14] Improvements in peak vertical force,[11,12] as well as subjectively assessed evidence of lameness,[12] and some physical activities[13] were found in dogs that received omega-3 fatty acid–enriched diets, but not control diets, in various studies. Results of 1 study[14] revealed that the omega-3 fatty acid–enriched diet was associated with a more rapid decrease in carprofen dosage for signs of lameness in osteoarthritic dogs, compared with that for dogs fed a control diet. Omega-3 fatty acids, especially EPA and DHA, reduce PGE_2, cyclooxygenase-2, IL-1α, and tumor necrosis factor-α expression and aggrecanase enzyme production from chondrocytes.[15] Taken together, the results of the described investigations suggest that dietary supplementation with EPA and DHA following TPLO in dogs may slow progression of osteoarthritis in the affected joint.

The proinflammatory factors PGE_2 and IL-1β have central roles in the development and progression of osteoarthritis and are considered useful indicators of inflammation in joints affected by osteoarthritis or CCL disease in dogs.[16–23] The effects of an omega-3 fatty acid–enriched diet and rehabilitation on synovial fluid IL-1β concentrations are unknown. Prostaglandin E_2 is also important in bone metabolism and healing,[24,25] and decreased PGE_2 concentrations resulting from the supplemental administration of omega-3 fatty acids may reduce osteoarthritis progression,[26] but could also compromise healing of the osteotomy site after TPLO.[24]

Patellar desmitis has also been reported[27–29] as a sequela to TPLO and is characterized on radiographs by soft tissue swelling, patellar ligament thickening, and irregular patellar ligament margins. These findings can be associated with clinical signs, including evidence of pain on palpation and lameness.[28,29] Patellar ligament thickening is often self-limiting in nature, although conservative treatment with NSAIDs and leash-controlled activity for 6 to 8 weeks has been recommended in clinical cases.[27,28]

Recently, our group investigated clinical outcomes (including ground reaction force, tibial plateau angle, and limb circumference measurements; subjective pain and lameness scoring by surgeons and dog owners; and daily activity recorded by accelerometry) for dogs that received an omega-3 fatty acid and protein–enriched food (TF)[a] or a CF formulated for maintenance of adult dogs[b] alone or in combination with physical rehabilitation after TPLO.[30] In that study,[30] feeding the TF was associated with greater peak vertical force and vertical impulse, and rehabilitation was associated with greater peak vertical force and more time spent in light-to-moderate activity, compared with results for dogs not receiving these treatments. In addition, owner assessments indicated lower frequencies of lameness and signs of pain during some activities for the TF group, compared with other groups, and for the TF-R and CF-R groups, compared with the CF group. In parallel with that study,[30] we prospectively investigated whether the TF and rehabilitation program would affect stifle joint inflammation and osteoarthritis progression following TPLO. The objective of the study reported here was to assess synovial fluid PGE_2 and IL-1β concentrations, radiographic evidence of osteoarthritis progression, osteotomy healing time, and incidence of patellar desmitis in the affected limbs of the dogs in the aforementioned study.[30] We hypothesized that the TF diet and physical rehabilitation program would each be associated with lower synovial fluid concentrations of PGE_2 and IL-1β, slower progression of osteoarthritis, delayed healing of the osteotomy site, and lower incidence of patellar desmitis, compared with findings for dogs not receiving these treatments.

Materials and Methods

Dogs, treatment groups, and blinding

Adult dogs > 1 year of age were prospectively recruited for study inclusion between August 1, 2010, and February 1, 2014. All dogs enrolled underwent TPLO and stifle joint arthroscopy for acute CCL disease and were randomly assigned to receive 1 of 4 postoperative treatments: a dry omega-3 fatty acid and protein–enriched dog food formulated to support joint health (TF),[a] a dry food formulated for maintenance of adult dogs (CF),[b] TF-R, or CF-R as described elsewhere.[30] The TF contained 31% crude protein with 1.03% omega-3 fatty acids (0.69% EPA and DHA) and glucosamine (1,100 mg/kg) added (values reported as minimum content on an as-fed basis), whereas the CF contained 21% crude protein without added omega-3 fatty acids or glucosamine.[30]

Exclusion criteria included signs of systemic illness, CBC or serum biochemical analysis results outside of the laboratory reference ranges, signs of CCL disease in the affected limb ≥ 2 months prior to surgery, or CCL disease of the contralateral limb. Dogs were also excluded if there was any concurrent or previous history of immune-mediated joint disease or osteochondrosis dissecans of the pelvic limbs, patellar luxation, or fractures of the distal aspect of a femur or proximal aspect of a tibia. Signs of joint disease in the forelimbs, administration of corticosteroids ≤ 2 weeks before surgery, or administration of an NSAID ≤ 24 hours before surgery were also causes for exclusion.

Study procedures were approved by the Institutional Animal Care and Use Committee of Oregon State University. Owners provided informed consent prior to enrollment of dogs. Investigators, dog owners, and veterinarians involved in case management were blinded to the food that was fed to the dogs. Radiologists who performed osteoarthritis scoring and assessments for osteotomy healing and patellar ligament thickness were blinded to all treatment group assignments.

Surgical procedures

Arthroscopic and TPLO procedures were performed in dogs under general anesthesia as previously described,[30–32] with the day of surgery considered day 0 and data obtained prior to surgery on day 0 used as baseline values. The medial meniscus of the affected stifle joint was inspected by arthroscopy and categorized as intact or as having radial tears, a longitudinal (ie, bucket-handle) tear, or severe disease (multiple bucket-handle tears or displacement or loss of meniscal tissue).[30,32] Bucket-handle tears or severe meniscal disease were treated by partial caudal meniscectomy and caudal hemimeniscectomy, respectively.[32] Meniscal release was not performed in any dog. Dogs received 1 dose of carprofen[d] (4.4 mg/kg [2 mg/lb], SC) at the time of surgery but at no other time during the course of the study. Cefazolin[e] (22 mg/kg [10 mg/lb], IV) was administered 30 minutes before surgery and every 90 minutes during the procedure. Postoperative analgesia was provided with hydromorphone hydrochloride[f] (0.1 mg/kg [0.045 mg/lb], IV, q 4 to 6 h) for 24 to 48 hours, followed by sustained-release morphine sulfate[g] (1 mg/kg [0.45 mg/lb] PO, q 12 h) for 7 days. Any dog that developed lameness as reported by the owner during the study period was evaluated by the researchers. Dogs with confirmed lameness were removed from subsequent data analysis and further enrollment in the study.

Diets and physical rehabilitation

The postoperative feeding and rehabilitation protocols were described elsewhere.[30] Briefly, dogs were fed the assigned foods (TF or CF) beginning with the first meal after TPLO, and the food was supplied to owners in unmarked bags throughout the study. Owners were instructed to feed the same volume of dry food as provided before the study and to refrain from administration of NSAIDs, homeopathic treatments, vitamins, nutraceuticals, or other supplements to their dogs during the study. The assigned food was to comprise ≥ 90% of the dog's diet.

Owners of dogs in all treatment groups were instructed to perform passive range-of-motion exercises on the treated limb (15 repetitions, 2 times/d) from 3 days to 3 months after surgery. All dogs were allowed leash walks twice daily for 5 to 10 minutes beginning 2 weeks after surgery. Beginning 8 weeks after surgery, the length of the walks was increased by 10 min/walk/wk until 3 months after surgery, when dogs were allowed any activity without restrictions.

In addition to walking and range-of-motion exercises, owners of dogs in the TF-R and CF-R groups were instructed to have their dogs perform sit-to-stand exercises (10 repetitions, 3 times/d) from 2 weeks until 3 months after surgery. Beginning 3 to 4 weeks after surgery, dogs in these 2 groups received underwater treadmill treatments (once weekly for 3 weeks, then twice weekly for 2 weeks, and then 3 times weekly for 3 weeks) according to a predetermined protocol.[30] The water level was initially set at approximately the mid-to-distal tibial region and was subsequently raised by predetermined increments at the start of each session until a maximum height at the level of the shoulder (ie, at the level of the scapulohumeral joint) was reached. The treadmill speed (range, 1.3 to 1.6 km/h [0.8 to 1.0 mile/h]) was set to allow dogs to walk smoothly but not trot or pace. Time on the treadmill began with 5 minutes at the first session and was increased by up to 5 minutes at each subsequent session until dogs spent a maximum of 30 min/session on the treadmill. Adjustments were determined by a certified canine rehabilitation practitioner, and the time required to reach maximum water height and treadmill time varied among patients. No other treatments were performed during the treadmill sessions.

Body weight and body condition score (scale of 1 [extremely thin] to 9 [obese][33]) of the dogs were evaluated immediately prior to surgery and 8 and 24 weeks after surgery, and owner compliance with management instructions was assessed by 1 investigator (SSO) as described.[30]

Synovial fluid biomarkers of inflammation

Synovial fluid samples were collected from each dog at baseline (immediately prior to surgery) and while sedated for radiography 8 weeks and 24 weeks later. Briefly, hair over the affected stifle joint was clipped, and the site was aseptically prepared and draped. A 22-gauge needle and 3-mL syringe were used to draw synovial fluid from the joint at a region 2 to 3 cm proximal to the tibial crest and 0.5 to 1 cm medial or lateral to the patellar ligament. Synovial fluid (0.4 to 2.0 mL) was immediately placed in microcentrifuge tubes and frozen at –80°C until testing.[34]

Synovial fluid PGE_2 concentrations were analyzed with a commercially available monoclonal, antibody-based, competitive enzyme-linked immunoassay.[h] Antibody cross-reactivity had been previously confirmed in canine synovial fluid.[35] The preliminary assay validation for this study was performed with equine synovial fluid owing to the volume of synovial fluid required for validation procedures. These samples were obtained from the stifle joints of adult horses immediately following euthanasia for reasons unrelated to joint disease and were stored at –80°C in the laboratory of one of the authors (WIB). Assay linearity, effect of various extraction techniques, spiking recovery, and dilutional parallelism (dilutions ranging from 1:10 to 1:100) were assessed. Results (not shown) showed acceptable dilutional parallelism and spiking recovery and revealed no interference with assay performance following serial dilution or in response to different extraction techniques. These findings were repeated by testing serial dilutions of a small number of canine test samples, and sample extraction was not carried out before analysis of canine samples. All canine study samples were diluted at an initial ratio of 1:10 in assay buffer, and standards were prepared with a 1:10 dilution of equine synovial fluid in assay buffer for all assays (to provide similar sample matrix effects in samples and standards). Interassay variation was 6.00%, and intra-assay variation was 18.85% for the 15.625 pg/mL (low concentration) and 4.27% for the 250 pg/mL (high concentration) standards.

Assessment of synovial fluid IL-1β concentrations was performed with a commercially available enzyme-linked immunoassay[i] developed and marketed for use with canine synovial fluid.[36] The test was performed according to the manufacturer's directions.

Radiographic assessments

All dogs underwent radiography of the affected limb at baseline (immediately before surgery), immediately after surgery, and 8 and 24 weeks later. Dogs were sedated for radiography with a combination of dexmedetomidine[j] (5 µg/kg [2.27 µg/lb], IV) and butorphanol[k] (0.1 mg/kg, IV) except for immediate postoperative imaging. Acceptable reduction without signs of translation or a cranial osteotomy gap > 1 to 2 mm was verified radiographically on immediate postoperative images for all dogs.

The tibial plateau angle was measured as previously described.[31] Board-certified surgeons (WIB and JJW) performed these assessments by examination of mediolateral radiographic images.

For osteoarthritis scoring, mediolateral and craniocaudal projections of the affected stifle joint were evaluated by 2 board-certified radiologists, and the mean score from the 2 observers was determined for each dog at each time point. The osteoarthritis scoring system used was modified from human and canine osteoarthritis grading scales described previously.[37–39] Radiographs (both mediolateral and craniocaudal views) were graded according to 2 scales. The first scale generally characterized the degree of osteoarthritis as follows: 0 = no abnormalities noted; 1 = joint effusion, loss or degeneration of articular cartilage, or both present, with no osteophytosis and no bone sclerosis; 2 = subchondral bone sclerosis, minimal osteophytosis, or both present (in addition to signs seen for a score of 1); 3 = moderate to severe osteophytosis or intra-articular mineralization present (in addition to the signs seen for scores 1 and 2) with no bone cysts; and 4 = appearance of subchondral cysts (in addition to signs seen in scores 1, 2, and 3). Once the first score was assigned, the radiologist then adjusted that score according to the overall severity of signs observed (treated as a subdivision of the first score). This meant that each score had a subscore assigned between 0 and 0.75, where 0 = normal to minimal, 0.25 = mild, 0.5 = moderate, and 0.75 = severe. The subscore was then added to the first score. For example, a score of 1 was assigned if joint effusion and loss of articular cartilage were observed without other changes. If the radiologist then deemed the joint effusion and articular cartilage loss were moderate, the score would be adjusted to 1.5. If the joint effusion and articular cartilage loss were severe, the adjusted score would be calculated as 1 + 0.75 = 1.75.

Tibia osteotomy healing and patellar ligament assessments

Tibia osteotomy healing was assessed by consensus of 2 board-certified radiologists at the same 4 time points as osteoarthritis scoring. Craniocaudal and mediolateral projections were examined for this evaluation, and the amount and quality of the callus and the presence of a fracture line were determined. A healed fracture was defined as complete isoopaque bridging of ≥ 2 cortices.[40] Healing was recorded as present or absent at each evaluation.

Patellar ligament thickening was assessed by 1 individual (VDV) who examined the affected stifle joint. The radiographic ligament thickness was measured at 75% of the distance from its origin on the patella to its insertion on the tibial tuberosity as described elsewhere.[20,41]

Statistical analysis

Potential confounders identified at the start of the study included age, sex, breed, body weight, body condition score, owner-reported compliance, whether meniscal surgery had been performed, tibial plateau angle, and whether CCL disease developed in the contralateral limb. Continuous variables were tested for normal distribution with the Shapiro-Wilk test. Identified potential confounders were assessed by comparison between groups by 1-way ANOVA when data were normally distributed (age, body weight, and tibial plateau angle) and by the Kruskal-Wallis test when data were not normally distributed (body condition score).

The models applied to the data estimated the variation in each variable accounted for by time (weeks) after surgery, within-dog variability, whether rehabilitation was performed (yes vs no), diet (TF vs CF), and treatment-by-time interaction. The minimal adequate model for each response variable was determined by stepwise regression. Following derivation of the minimal adequate model, a multi-way ANOVA or Kruskal-Wallis test was used to assess data that were and were not consistent with a normal distribution, respectively. False discovery rate was controlled with the Benjamini-Hochberg method to derive adjusted *P* values. Posttest analysis of between-group differences (ie, assessment of the effects of time after surgery, diet type, rehabilitation treatment, and interactions among time, diet, and rehabilitation treatment) was performed with the Tukey honest significant difference test following the multi-way ANOVA or Dunn test and the Kruskal-Wallis test. Values of $P < 0.05$ were considered significant. Statistical analyses were performed in an open-source statistical programming environment.[42,l]

Results

Signalment and demographic data for dogs in this study were previously reported.[30] Twenty-eight spayed females, 18 neutered males, and 2 sexually intact males were included. The sexually intact males were in the CF and TF-R groups, and there was no significant ($P > 0.05$) difference in the proportions of spayed females and neutered males among groups. Ages ranged from 1 to 10 years, and mean ± SD age did not differ significantly ($P > 0.05$) among groups. There were 13 individual breeds represented in the study, most commonly Labrador Retriever (n = 9), Golden Retriever (6), and American Staffordshire Terrier (3). Eighteen dogs were of mixed breeds.

Body weight, body condition score, and tibial plateau angle data were also reported elsewhere.[30] These results and differences among the TF, CF, TF-R, and CF-R groups were summarized **(Table 1)**.

Owners reported that they followed exercise, activity, feeding, and postoperative care instructions adequately when asked verbally at each time point or physical rehabilitation session; however, 3 of 48 (6%) dogs, including 1 in the CF group and 1 each in the TF and TF-R groups, did not complete the study. All 3 dogs were removed because of dietary compliance issues (refusal or inadequate consumption of the food).

The incidence and severity of meniscal disease at the time of surgery did not differ significantly ($P > 0.05$) among the 4 treatment groups. These findings and the incidence of complications that developed during the study[30] were summarized **(Table 2)**. Overall, complications after surgery were rare (6/48 [13%]). Five dogs that developed contralateral CCL disease or postopera-

Table 1—Comparison of results (mean ± SD) for variables of interest for 48 client-owned dogs in a study to evaluate the effects of diet and physical rehabilitation on radiographic findings and markers of synovial inflammation following TPLO and arthroscopic surgery for treatment of CCL disease.

Variable	TF	CF	TF-R	CF-R
Body weight (kg)				
Baseline	38.5 ± 10.6	39.2 ± 8.2	34 ± 10.5	37.4 ± 12.6
8 wk	38 ± 10.7	36 ± 7.5	33 ± 11.2	34.3 ± 12.5
24 wk	37.7 ± 11.2	36.4 ± 9.4	34.7 ± 12.9	34.1 ± 12.4
Body condition score*				
Baseline	5.8 ± 1.2	5.8 ± 1.1	5.1 ± 0.3	5.8 ± 1.4
8 wk	5.8 ± 1.4	5.1 ± 0.3	5.5 ± 1.1	5.8 ± 1.2
24 wk	5.6 ± 1.4	6.0 ± 1.4	5.8 ± 1	5.5 ± 1.3
Tibial plateau angle (°)†				
Baseline	25.7 ± 3.7	25.5 ± 3.4	24.8 ± 3.3	26.8 ± 4.5
Immediately after surgery	8.0 ± 3.4	6.2 ± 2.9	9.5 ± 5.5	7.4 ± 3.2
8 wk	7.8 ± 3.9	6.2 ± 2.4	8.9 ± 2.9	7.3 ± 3.3
24 wk	7.4 ± 3.0	6.4 ± 2.6	9.1 ± 3.3	8.7 ± 6.0
Synovial fluid PGE_2 concentration (pg/mL)				
Baseline	1,189.37 ± 1,134.06	1,085.67 ± 814.54	964.31 ± 996.07	2,031.93 ± 2,460.43
8 wk	1,007.63 ± 911.06	2,222.47 ± 1345.99	652.53 ± 269.50	1,523.96 ± 1,073.25
24 wk	672.63 ± 801.32	1,360.57 ± 1127.39	686.77 ± 414.90	713.70 ± 487.88
Synovial fluid IL-1β concentration (pg/mL)				
Baseline	0.07 ± 0.04	0.09 ± 0.06	0.19 ± 0.30	0.11 ± 0.14
8 wk	0.12 ± 0.12	0.12 ± 0.14	0.16 ± 0.24	0.10 ± 0.10
24 wk	0.11 ± 0.10	0.08 ± 0.04	0.11 ± 0.11	0.10 ± 0.10
Patellar ligament thickness (cm)				
Baseline	0.31 ± 0.03	0.32 ± 0.06	0.33 ± 0.07	0.29 ± 0.07
Immediately after surgery	0.45 ± 0.15	0.42 ± 0.10	0.52 ± 0.11	0.59 ± 0.21
8 wk	0.62 ± 0.20	0.68 ± 0.22	0.61 ± 0.25	0.77 ± 0.27
24 wk	0.60 ± 0.17	0.59 ± 0.22	0.52 ± 0.20	0.58 ± 0.23
Osteoarthritis score†				
Baseline	1.6 ± 0.5	1.6 ± 0.4	1.3 ± 0.4	1.3 ± 0.6
8 wk	1.8 ± 0.5	1.7 ± 0.5	1.3 ± 0.4	1.5 ± 0.7
24 wk	1.4 ± 0.5	2.1 ± 0.4	1.6 ± 0.4	2.0 ± 0.5
Proportion of dogs with healed tibial osteotomy†				
8 wk	6/11	5/11	1/11	5/12
24 wk	11/11	11/11	9/11	12/12

Dogs were randomly assigned to receive a dry omega-3 fatty acid and protein–enriched dog food formulated to support joint health (TF), a dry food formulated for maintenance of adult dogs (CF), TF-R, or CF-R after surgery. Baseline values were determined prior to surgery on day 0 for all dogs (n = 12/group). Removal of dogs from the study caused changes in group size at 8 weeks in the TF (n = 11), CF (11), and TF-R (11) groups and at 16 and 24 weeks in the TF (10) TF-R (10), CF (9), and CF-R (11) groups.

*Assessed on a scale of 1 (extremely thin) to 9 (obese).[33] †Values determined radiographically. Osteoarthritis scores were determined by 2 board-certified radiologists. The osteoarthritis scoring system used was modified from human and canine osteoarthritis grading scales described previously (minimum and maximum possible scores, 0 and 4, respectively).[38–40] Body weight, body condition score, and tibial plateau angle data were previously reported.[30]

Table 2—Descriptive summary of arthroscopically identified meniscal injuries at the time of surgery and complications that developed after TPLO and arthroscopic surgery[30] for the same 48 dogs as in Table 1.

Variable	TF	CF	TF-R	CF-R
Medial meniscus injury at surgery				
Radial tears	5	6	3	7
Bucket-handle tears	4	2	3	2
Severe disease	1	3	1	2
Postoperative complications				
Incisional inflammation and serosanguinous discharge*	0	1	0	0
Medial meniscal disease (bucket-handle tears) after surgery†	0	1	1	0
CCL disease of the contralateral limb†	1	1	0	1

Medial meniscal disease was identified and treated arthroscopically; the meniscus was categorized as intact (with no signs of disease) or as having radial tears, a longitudinal (ie, bucket-handle) tear, or severe disease (double or triple bucket-handle tears or displacement or loss of meniscal tissue).

*Results of aerobic and anaerobic culture were negative. †Dogs that developed meniscal injury in the affected joint (16 to 20 weeks after surgery; confirmed and treated in a second arthroscopic procedure) or contralateral CCL disease (20 to 24 weeks after surgery) were removed from subsequent data analysis.

tive medial meniscal disease were removed from the remaining data analyses starting on the date they developed the complication. The remaining dog had incisional complications and was not removed from the study, but a 5-day delay in the start of increased exercise was instituted to allow for wound healing, which was uneventful.

Mean synovial fluid PGE_2 concentrations were similar among groups at baseline immediately prior to surgery ($P > 0.05$) and decreased significantly in all groups over the 24 weeks after surgery ($P < 0.01$; Table 1). Diet was significantly ($P < 0.01$) associated with this variable, with dogs receiving CF having significantly higher PGE_2 concentrations on postoperative evaluations than dogs receiving TF. There was no significant ($P > 0.05$) association of rehabilitation with this variable. No significant ($P > 0.05$) differences in synovial fluid IL-1β concentrations were identified at any time in any groups, and neither diet nor rehabilitation status was significantly associated with IL-1β concentration.

Mean osteoarthritis scores did not differ significantly ($P > 0.05$) among groups prior to TPLO (Table 1). A score of 4 was not assigned to any of the dogs during the study (ie, none had radiographic evidence of subchondral bone cysts). Osteoarthritis progression was identified in all 4 groups over time, and a significant ($P < 0.05$) interaction between time and treatment was identified for this variable. Dogs of the TF and TF-R groups had lower osteoarthritis scores (less osteoarthritis progression) than did dogs of the CF and CF-R groups at 24 weeks after surgery, and dogs of the TF-R group had significantly ($P = 0.012$) lower scores over time, compared with the other 3 groups. Dogs that underwent rehabilitation (TF-R and CF-R groups) also had significantly ($P = 0.012$) less progression of osteoarthritis over time than dogs that did not.

Patellar ligament thickness increased in all groups over time after surgery ($P < 0.01$ for all comparisons). There was no significant association between diet or rehabilitation and patellar ligament thickening.

Diet was significantly ($P < 0.001$) associated with osteotomy site healing, with smaller proportions of dogs receiving TF having complete healing of the osteotomy site on radiographic examinations at 8 and 24 weeks, compared with dogs receiving CF. There was no significant ($P > 0.05$) association between rehabilitation status and this variable (Table 1).

Discussion

Dietary supplementation with omega-3 fatty acids rich in EPA and DHA results in higher cell membrane content of omega-3 and lower amounts of omega-6 fatty acids.[13,14,43,44] The result is reduced production of arachidonic acid (an omega-6 fatty acid) and proinflammatory mediators, including PGs such as PGE_2, leukotrienes, and thromboxanes of the 2 and 4 series.[13,14,43,44] Instead, there is an increase in 3-series PGs and 5-series leukotrienes, which are less proinflammatory.[14,44,45] Addition of omega-3 fatty acids to a diet may also reduce serum expression and activity of cyclooxygenase-2 and expression of proteoglycan-degrading enzymes and synovial matrix metalloproteinases.[15,46]

The total omega-3 fatty acid content in foods or supplementary capsules does not necessarily indicate the EPA or DHA content provided to dogs because 1 fatty acid (α-linoleic acid) is not efficiently converted to EPA or DHA in this species.[47] The reported therapeutic dose of EPA and DHA for the management of osteoarthritis in dogs is between 230 and 370 mg/kg body weight$^{0.75}$ per day, regardless of the total amount of omega-3 fatty acids consumed.[26] As previously described,[30] the TF in the present study contained manufacturer-reported amounts of EPA and DHA that, in feeding an amount of diet sufficient for the energy requirements of a dog, would achieve the combined daily dose of EPA and DHA expected to have a therapeutic effect.

Both PGE_2 and IL-1β are involved in the progression of osteoarthritis; PGE_2 has been shown to increase sensitization of nociceptors, stimulate new blood vessel formation, induce expression of matrix metalloproteinases, and regulate proliferation of chondrocytes and synthesis of extracellular membrane components.[17,18,22,48,49] The synovial fluid concentration of PGE_2 was found to be positively correlated with the degree of lameness in dogs with osteoarthritis induced by CCL transection.[19] In addition to its role in the acute phase of inflammation, PGE_2 also has a predominant role in bone remodeling and healing.[25,50–52] In the overall modeling, dogs that received the TF in the present study had significantly lower synovial fluid PGE_2 concentrations in the affected stifle joint over time after surgery than did dogs that received the CF, regardless of whether they received physical rehabilitation. The lower PGE_2 concentrations in dogs receiving the TF might have contributed in part to the previously reported[30] finding that mean ground reaction forces are significantly greater in dogs of the TF-R group than those of other groups at these same time points. In the present study, the lower PGE_2 concentrations might have had a role in reduced progression of osteoarthritis in dogs that received the TF as indicated by significantly lower postoperative radiographic osteoarthritis scores over time than in dogs receiving the CF.

Interleukin-1β plays a central role in the pathophysiology of osteoarthritis in people by inducing increased activity of proteolytic enzymes involved in cartilage degradation and increasing expression of inflammatory mediators and cell infiltration; this cytokine also suppresses cartilage anabolism and induces chondrocyte apoptosis.[21] Studies[20,23] evaluating proinflammatory cytokines in synovial fluid of dogs with naturally acquired CCL disease found significantly higher concentrations of IL-1β, tumor necrosis factor-α, and other cytokines in dogs with CCL disease than in control dogs without the condition. Because only small amounts of synovial fluid were collected in the present study, only IL-1β and PGE_2 were measured, and this was an important limitation of the study. Subchondral bone cysts are rare in dogs with osteoarthritis, and none of the dogs in our study received a maximum osteoarthritis score of 4; thus, the osteoarthritis scoring scale used was effectively identical to the scale reported by Innes et al[39] in which the most severe degree of osteoarthritis was assigned a score of 3.

Diet was not significantly associated with synovial fluid IL-1β concentrations in dogs of the present study. We measured IL-1β concentrations at baseline (ie, immediately prior to surgery) and 8 and 24 weeks after surgery; it is possible that differences could have occurred at other time points or that synovial fluid IL-1β was not affected by dietary supplementation with DHA and EPA. Osteoarthritis scores increased in all groups over time, and previous research[21,36,48,49] has indicated that Il-1β might be directly involved in progression of osteoarthritis. Considering that radiographic evidence of osteoarthritis progression was measurable in all groups, despite lesser progression in dogs receiving the TF, we speculated that this could have been related in part to the continued presence of unchanging synovial fluid IL-1β concentrations.[21]

Prostaglandin E_2 is additionally involved in bone metabolism and in various stages of bone healing.[50] During the inflammatory phase of bone healing, proinflammatory cytokines and growth factors increase the production of PGE_2, leading to high concentrations at the fracture callus during the first 2 weeks after injury.[25] The PGE_2 increases the replication and differentiation of osteoblastic and osteoclastic cells, thereby stimulating bone formation, resorption, and remodeling.[51,52] The lower PGE_2 concentrations attributed to the effects of omega-3 fatty acids in the TF of the present study might explain the slower tibial osteotomy healing identified in dogs that received this food, compared with dogs that received the CF (regardless of rehabilitation status). We previously found[30] that these same TF-fed dogs had greater peak vertical force and vertical impulse and improved case-specific outcome measures after surgery, compared with CF-fed dogs. None of the study dogs sustained implant failure or postoperative derotation of the tibial plateau in the 6 months following surgery, regardless of the treatment they received. These findings suggested that the slower osteotomy healing observed in dogs fed the TF might not have any clinical impact in healthy dogs following TPLO and arthroscopic surgery. Standard radiographic evaluation of bone healing may, however, be recommended at 12 weeks instead of 8 weeks after surgery in these dogs, considering that diet alone was associated with slower healing time (only 7/22 dogs in TF-fed groups had complete osteotomy healing at 8 weeks).

Postoperative physical rehabilitation was significantly associated with slower progression of osteoarthritis on the basis of radiographic scores following TPLO and arthroscopic surgery, regardless of diet, in the present study. Rehabilitation was not significantly associated with synovial PGE_2 or IL-1β concentrations, patellar ligament thickness, or rates of osteotomy healing in the dogs of this study; this, together with data from our previous study,[30] supported findings that use of a rehabilitation protocol including underwater treadmill exercise as described herein after TPLO and arthroscopic surgery is unlikely to have deleterious effects and may contribute to improvements in some measures of short-term (6 months) clinical outcome, such as activity level.

Anecdotally, some surgeons have been hesitant to recommend rehabilitation after TPLO surgery because of concerns that patellar desmitis might result from overactivity. Although patellar ligament thickness increased in all groups over time, the present study found no significant association between this variable (which is 1 indicator of patellar desmitis) and diet or rehabilitation. This result suggested that the rehabilitation protocol used does not increase the risk for patellar desmitis; however, it was possible that these results represented a type II error. The clinical importance of patellar desmitis is difficult to ascertain because the condition is associated with a low frequency of clinical signs (pain on palpation and lameness).[27–29] No clinical evidence of patellar desmitis was found in the dogs of this study, despite the radiographic evidence of patellar ligament thickening. Thus, the clinical relevance of the finding was unknown.

Potential limitations of the study included small group sizes, enrollment of various breeds, reliance on owner-supplied information regarding duration of lameness prior to enrollment and compliance with at-home protocols during the study, and inability to control for potential confounders. More chronic inflammation in some dogs might have impacted synovial fluid PGE_2 and IL-1β concentrations. Because caloric content of the 2 foods differed, the need for blinding of researchers and owners to food assignment led to the decision that owners would be instructed to give the same amount of food they had fed previously; however, the subjective owner decisions regarding the amount of food needed were considered to reflect a typical clinical situation. Although owners were asked not to provide dog treats containing added omega-3 fatty acids or other supplements, it is possible some dogs received such products, and this could have influenced the results. As previously reported,[30] the amounts of omega-3 fatty acids (including DHA and EPA concentrations) and glucosamine in the CF were not known, and although none of the listed ingredients were known to contain substantial amounts of these products, this might have represented a study limitation. Finally, although radiologists were blinded to group assignments of the dogs, interobserver variability was not assessed for osteotomy site healing and osteoarthritis scores.

The results of the present study suggested that feeding of an omega-3 fatty acid and protein–enriched food such as the TF, with or without a physical rehabilitation program, might benefit dogs following TPLO and arthroscopic surgery by resulting in lower synovial fluid PGE_2 concentrations and slowing the progression of osteoarthritis in the 6 months following surgery, compared with results for dogs fed a maintenance food such as the CF. Our results indicated that postoperative rehabilitation may also slow progression of osteoarthritis in dogs following TPLO, and from the findings of this and our previous study,[30] the protocol used did not appear to negatively impact other recovery variables following surgery. Although feeding the TF in this study was associated with slower tibial osteotomy healing, we subjectively observed no evidence of complications resulting from prolonged healing time, and this suggested that the finding was of low clinical relevance. However, further investigation of this aspect may be warranted. Future studies of the effects of an omega-3 fatty acid and protein–enriched diet and rehabilitation in dogs following surgery should include a larger number of dogs and potentially dogs with joint diseases other than CCL injury.

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Footnotes

a. Purina JM Joint Mobility Diet, Nestlé Purina PetCare, St Louis, Mo.
b. Pedigree Adult Complete Nutrition, MARS Inc, McKean, Va.
c. DePuy Synthes Vet, West Chester, Pa.
d. Rimadyl, Zoetis Inc, Kalamazoo, Mich.
e. Cefazolin for injection, HIKMA Farmaceutica SS (Portugal), distributed by West-Ward Pharmaceuticals Corp, West Eatontown, NJ.
f. Hydromorphone hydrochloride injection, West-Ward Pharmaceuticals Corp, Eatontown, NJ.
g. Morphine sulfate extended release, Mallinckrodt Inc, Hazelwood, Mo.
h. PGE_2 Express ELISA kit, Cayman Chemical Co, Ann Arbor, Mich.
i. GSI canine IL-1 beta ELISA kit-synovial fluid, Genorise Scientific, Glen Mills, Pa.
j. Dexdomitor, Orion Pharma, Orion Corporation (Finland), distributed by Pfizer Animal Health, New York, NY.
k. Torbugesic, Pfizer Inc, New York, NY.
l. R, version 3.3.3, R Foundation for Statistical Computing, Vienna, Austria. Available at: www.R-project.org. Accessed Nov 30, 2014.

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